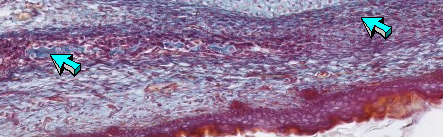
**BONE DEVELOPMENT**

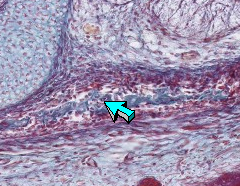
There are two types of bone formation: intramembranous and intracartilaginous (endochondral) ossification. Membrane bones (those principally formed by intramembranous bone formation) are the flat bones of the skull: i.e., frontal, parietal, occipital. Parts of the mandible and clavicle are formed by the intramembranous type of formation as well as parts by endochondral ossification. These bones are sometimes called mixed bones. Endochondral bone formation takes place in the long and short bones of the body, that is, those which are used primarily for support and locomotion.

**INTRAMEMBRANOUS BONE FORMATION**

[**[MCO 0016]**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf2_MCO0016&type=ax&section=bonedevelopment)Membranous Bone, Fetal Skull [**Java**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf2_MCO0016&type=jv&section=bonedevelopment) HIR-*frames 391-393, 396-397*



(Condensed mesenchyme and bone island)



(Trabeculae)



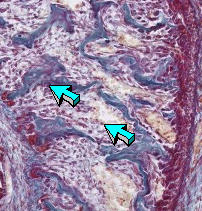
(Vascular Mesenchyme and Bone island)



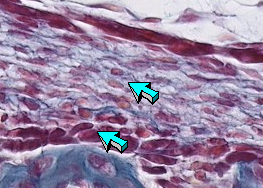
(Osteoblast = cuboidal and darker staining and Trabeculae)



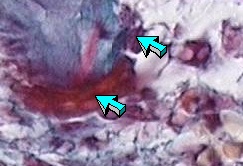
(Osteocyte)



(Trabecular/ spongey Bone and forming marrow)

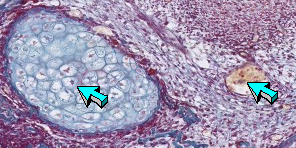


(Periosteum 🡪 Fibrous and osteogenic layers and osteoblasts)



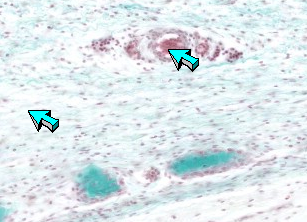
(Osteoclast = larger and Osteoblast = smaller)

\*\*\* As soon as you start forming bone, remodeling starts to occur)

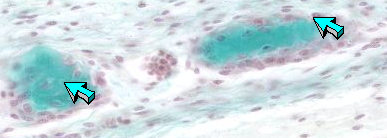


(Tooth and Cartilage)

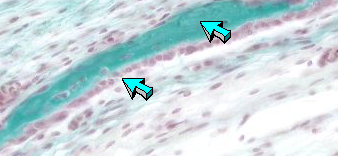
[**[UW 2006]**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf7_UW2006&type=ax&section=bonedevelopment)Membrane bone[**Java**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf7_UW2006&type=jv&section=bonedevelopment)



(Mesenchyme and blood vessel)



(Boney Island and Lining Osteoblasts)



(Trabeculae)

3 processes:

1. Intermembraneous Ossification
2. Appositional growth
3. calcification

[**[UW 2020]**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf7_UW2020&type=ax&section=bonedevelopment)Calvaria, Monkey[**Java**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf7_UW2020&type=jv&section=bonedevelopment)

 \*\*\*Endostial cells can be osteoprogenitor cells

**SEE DEMONSTRATION FOR ORIENTATION OF FETAL SKULL**

This section (**MCO 0016**) comes from a fetal rodent skull. The skull is oriented upside down with the �top� of the skull at the bottom of the section. The nasal cavity is characterized by open spaces with blue-staining cartilage conchae subdividing it. The oral cavity contains the muscular tongue at the top of the section.

The nasal cavity is characterized by the labyrinth of light blue-staining cartilage and space. Look for a layer of mesenchyme between the scalp and the light blue staining hyaline cartilage of the region over the nasal cavity. A hyaline cartilage bar (septum) separates the two large nasal cavities. The cartilage bar then runs laterally around the cavity. Lying between the cartilage and the skin at bottom of section is a region of condensed mesenchyme and forming bone. The earliest sign of **intramembranous bone formation** is the condensation of this mesenchyme. The mesenchymal cells coalesce to form a thickened sheet immediately underneath the scalp and dorsal to the nasal cavity or brain.

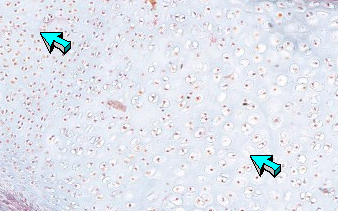
In the mesenchyme, look for a dark blue staining matrix. This represents the uncalcified bone matrix or osteoid. **You can not differentiate between calcified or uncalcified bone matrix because all mineral has been removed to allow sectioning of the tissue.** Moving more centrally (towards the middle of the ossification center), the process is more advanced with the formation of calcified bone matrix, first as small islands and then as a solid bar or sheet of light blue material.

Identify the osteoblasts lining the skin side of the bone. Osteocytes are the cells within or completely surrounded by the blue matrix. In a slightly more advanced stage, trabeculae are being formed by appositional growth. Identify the periosteum. There is no compact bone on the surfaces at this time as seen in the inner and outer tables of the adult skull bones (**UW 2020**). Bone marrow develops in the cavities between the trabeculae. Look for the large, acidophilic, multinucleated osteoclasts. When the bone tissue is first laid down, it is woven bone. The ossification center is where the process of bone formation first started for that particular bone. The center of ossification will be the most developed while the periphery will be the least developed with only small islands of matrix present. **UW 2006** is a section of skull bone that has started intramembranous ossification. Identify the greenish stained bony islands and trabeculae, osteocytes and osteoblasts. Can you find any osteoclasts?

**ENDOCHONDRAL BONE FORMATION**

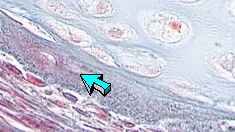
[**[MCO 0017]**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf2_MCO0017&type=ax&section=cartilageand%20bone)Fetal Finger, Early Endochond. Ossif. [**Java**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf2_MCO0017&type=jv&section=cartilageand%20bone)

HIR-*frames 398-399, 362, 405-408*



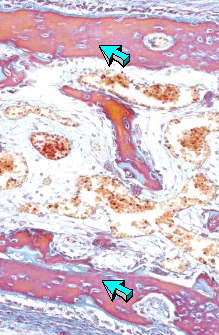
(Comparison to see hypertrophy of mesenchyme cells)

\*\*\*Hypertrophic cells secrete alkaline phosphotase

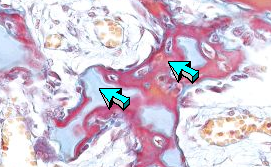


(Beginning of Periosteum)

[**[MCO 0617]**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf2_MCO0617&type=ax&section=cartilageand%20bone)Fetal Finger, Later Endochond. Ossif.[**Java**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf2_MCO0617&type=jv&section=cartilageand%20bone)



(Well Developed periostial collar)



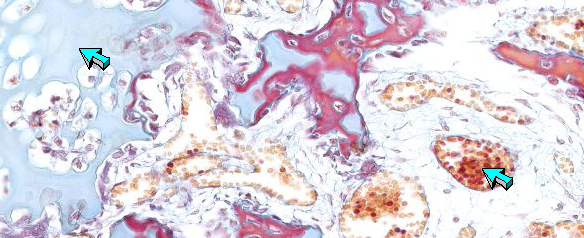
(Calcified matrix and trabecular bone)

\*\*\*blood vessels erode away uncalcified matrix and leave calfified matrix

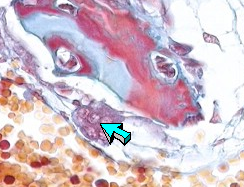
\*\*\*Blood vessels bring osteoprogenitor cells which migrate to surface of cavity where

uncalcified matrix has been eroded away….The Osteoprogenitor cells turn into

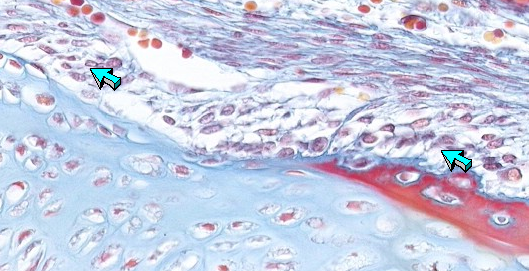
osteoblasts and lay down matrix



Matrix that will be eroded away and Blood vessel

(Osteoclast = multiple nuclei) (Osteocytes and osteoblasts)



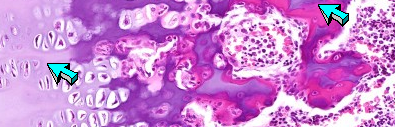
Perichondrium and Periosteum



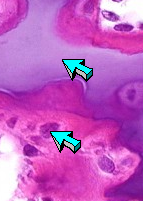
(Synovial Joint)

\*\*\*Synovial membrane begins to form

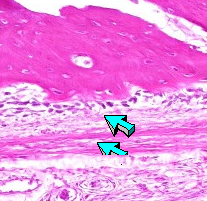
[**[LH 0026]**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf1_LH0026&type=ax&section=cartilageand%20bone) Fetal Finger [**Java**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf1_LH0026&type=jv&section=cartilageand%20bone)



(Hypertrophic Mesenchyme and primary spongiosa)

 (Osteoblast and calcified matrix)  (Osteoclast)

[**[SL 004]**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf4_SL004&type=ax&section=cartilageand%20bone) Fetal Digit (Endochondrol Oss.) [**Java**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf4_SL004&type=jv&section=cartilageand%20bone)

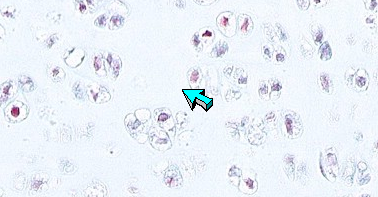
(Fibrous and Osteogenic layer of Periosteum) (Spicule/trabecula and Osteoblast)

In this developmental process, bone is formed *within* a pre-existing cartilage model. The cartilage must first be removed and then bone is laid down on remnants of calcified cartilage matrix. The first indication of the process is the hypertrophy of the hyaline cartilage cells in the midshaft of a long bone. These chondrocytes become enlarged and the matrix a lighter staining blue. This is where the primary ossification center is formed in all long bones (midshaft). At the same time, osteoprogenitor cells in the perichondrium begin laying down bone matrix to form a bone collar (red staining) around the outside of the cartilage model. This gives support during cartilage erosion and bone formation. The periosteal collar is formed within the connective tissue of the perichondrium/periosteum so it is formed by ***intramembranous ossification***.

Blood vessels and mesenchyme, called periosteal buds, enter through the bone collar into the forming primary ossification center in the midshaft of the bone. The periosteal buds erode away the degenerating cartilage. Osteoprogenitor cells, carried in by the invading blood vessels, migrate to the surface of the eroded calcified cartilage remnants where they differentiate into osteoblasts. Bone matrix is then laid down on remnants of calcified cartilage left within the eroded areas to form the first trabecula. The chondrocyte hypertrophy and blood vessel invasion then proceeds toward each end of the cartilage model of the bone. Look for some of these early stages of bone formation on **MCO 0017**. The cartilage cells in midshaft have hypertrophied, but no periosteal bud invasion has occurred from the bony collar that has formed in the perichondrium. In slide **MCO 0617** the process is further along with the invasion process now proceeding towards each end. A well developed bony collar, trabeculae, hypertrophied cartilage cells, erosion of cartilage and forming bone marrow are readily visible. Look for blood vessels entering the forming cavity through the bony collar. The remaining two slides, **LH0026** & **SL004**, are examples of later stages of the process as the epiphyseal plate begins to organize. The secondary ossification centers in long bones will form at each end within the epiphysis.

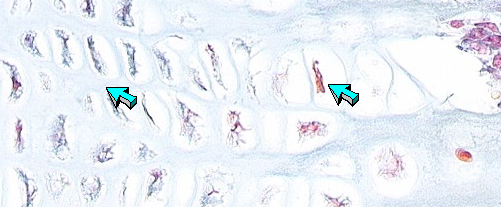
[**[MCO 0015]**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf2_MCO0015&type=ax&section=bonedevelopment) Joint, Human Fetus [**Java**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf2_MCO0015&type=jv&section=bonedevelopment) HIR-*frames 398-404*

 (Epiphyseal and Diaphyseal End)

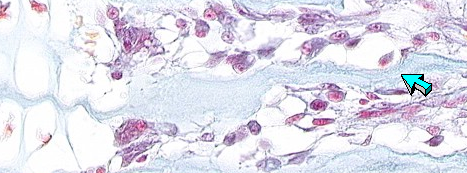
 (Resting Zone)



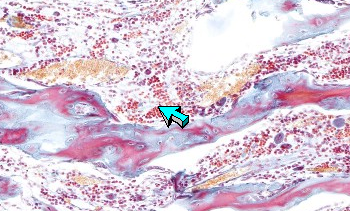
(Zone of Proliferation)



(Zone of hypertrophy and calcification)



(Resorption front and Zone of primary ossification)

 (Forming Marrow)

[**[MCO 0100]**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf2_MCO0100&type=ax&section=bonedevelopment)Developing Long Bone [**Java**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf2_MCO0100&type=jv&section=bonedevelopment)

In this later stage, the bone has been laid down in the center of the diaphysis and the process of degenerating cartilage has moved toward the ends and is more organized. Here it appears like the epiphyseal plate. The process in the epiphyseal plate and the primary ossification center is essentially the same, but the cartilagenous epiphyseal plate is organized into four well-defined regions. A series of stages may be seen illustrating the sequence of events as one moves from the ends of the cartilage model towards the primary ossification center.

Quiescent or Resting Zone: This is the normal hyaline cartilage near the epiphyseal end of the cartilage model. The chondrocytes are randomly scattered as in normal hyaline cartilage.

Proliferative Zone: a region of active mitosis of the cartilage cells. Here the cells form rows or columns of thin flat cells. The cell columns are oriented parallel to the long axis of the cartilage model. There is little matrix between the cells *within* the column, however, there is more matrix *between* the cell columns. This zone is where the mass of the cartilage is added by *interstitial growth* to increase the bone length. Each column is an isogenous group since they are all derived from one chondroblast.

The Zone of Maturation & Hypertrophy is characterized by enlarged cuboidal chondrocytes with decreased matrix between the cells. The cells in all these zones may be shrunken within their lacunae. Typically, this is a very short zone (1-3 cells).

The Zone of Calcifying Cartilage (Zone of Provisional Calcification) is the region where the chondrocytes are greatly enlarged and the amount of matrix between cells is greatly reduced. The matrix becomes heavily calcified and appears more basophilic. This zone is not well defined on the slide and is represented by the last few chondrocytes immediately adjacent to the resorption front. (Remember that all the mineralization is removed for sectioning.)

Some investigators add a fifth zone, the Zone of Invasion or Resorption. The Zone of Invasion or Resorption is a thin irregular line where cartilage cells and matrix are being removed by vascular buds which are invading the cartilage lacunae within the columns of cells. This is at the diaphyseal end of the cartilage columns. Longitudinal bars of calcified cartilage persist into the zone of primary ossification.

The Zone of Ossification, Primary Ossification Zone (primary spongiosa), a fifth zone listed in Gartner & Hiatt, is where woven bone is laid down by osteoblasts which differentiate from the osteoprogenitor cells of the vascular buds. *This is really not part of the epiphyseal plate.* The osteoblasts line the surface of the remnants of calcified cartilage. They lay down bone osteoid which rapidly calcifies. In this way, spicules or trabecula are formed with cores of calcified cartilage. Is the bone tissue laid down here, woven or lamellar bone? The new bone appears as red staining masses on the surface of blue staining calcified cartilage matrix.

In the more advanced stages, bone marrow has developed between the trabeculae. This would be closer to the center of the diaphysis. Look for large acidophilic osteoclasts on the surface of these trabeculae. These multinucleated cells are taking part in the important process of remodeling the bone. Look for any resorption cavities in this secondary zone of ossification (secondary spongiosa). The zone of secondary ossification is where the woven bone is being remodeled and replaced with lamellar bone. Once the epiphyseal plate closes, there can not be any increase in the length of that bone. Bones increase their diameter by *apposititional growth* of the periosteum laying down the outer circumferential lamellae. Therefore, *Intramembranous Ossification* increases the diameter of the bone.

Note that there is a difference between primary ossification zone and the primary ossification center.